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FEB 1 6 1946

The American Journal of Medical Technology

MAY, 1946

Vol. 12, No. 3

OFFICIAL PUBLICATION
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AMERICAN SOCIETY OF MEDICAL TECHNOLOGISTS
Published Bi-Monthly by The American Society of Medical Technologists
Printed by The Wines Printing Company
Business Office: Medical Center Bldg., Lafayette, La.
Editorial and Printing Office: 4310 W. Fort St., Detroit 9, Michigan

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The
AMERICAN JOURNAL
of
MEDICAL TECHNOLOGY

VOLUME 12 MAY, 1946 NUMBER 3

TECHNIQUE OF Rh TESTING

By ALEXANDER S. WIENER, M.D.

Head of the Blood Transfusion Division; Jewish Hospital, Brooklyn, N. Y.

Serologist to the Office of the Chief Medical Examiner of New York City.

Two varieties of human antisera are now available for Rh testing; that is, for determining whether a blood is Rh positive or Rh negative. One serum contains agglutinins (bivalent antibodies) while the other variety contains so-called glutinins or blocking antibodies (univalent antibodies)[1]. The technique for Rh testing differs depending upon the type of reagent available. For sera containing agglutinins, the agglutination technique is employed, while for sera containing glutinins or blocking antibodies, the so-called conglutination technique must be used. The essential difference between agglutination and conglutination can be summarized as follows:

Rh-positive red cells + Anti-Rh agglutinins (bivalent antibodies) → Agglutination

Rh-positive red cells + Anti-Rh blockers (univalent antibodies) + Conglutinin (X Protein) → Conglutination

Thus, agglutination proceeds in a single step. Each molecule of agglutinin being bivalent (in the chemical sense) links two red cells together, and in this way clumping of cells occurs in any isotonic medium. For the agglutination test, the most satisfactory medium is saline solution.

In the case of conglutinating sera, the antibody combines with the red cells but without clumping them. In the presence of whole plasma or serum, however, clumping will occur because plasma and serum contain the so-called protein or conglutinin which is absorbed onto the sensitized cells and makes them sticky, thus bringing about clumping.* While the clumping occurring in both methods looks alike microscopically, the clumps have several important differences. The technique giving best results for sera containing agglutinins will not work for sera containing univalent antibodies; on the other hand, the conglutination technique which must be used for sera containing univalent antibodies does not give as clear results with agglutinating sera. Therefore, it is essential to observe whether the serum you are using is labeled "Agglutinating Anti-Rh serum" or "Conglutinating Anti-Rh serum" and to use the appropriate technique depending on which serum is available.

Technique for Rh Testing Using Agglutinating Sera Only†

All blood suspensions must be fresh, and washed once by centrifuging and discarding the supernatant, the sediment being resuspended in enough fresh saline solution to make a 2 percent suspension (in terms of blood sediment). Mix one drop of the reagent† with a drop of the blood suspension (each drop .05 cc.) in a small narrow test tube (7 to 8 mm. inside diameter). Incubate the mixture for one hour in the water-bath at body temperature. Read reactions grossly by inspecting the sediments in each tube with the naked eye. Then rotate the tube gently at an angle in order to separate the sediment from the bottom of the tube. You may read the result with the naked eye. Also read miscroscopically under the low power by placing the tube on the stage under the objective

*Conglutinin is relatively thermostable but sensitive to even slight dilution by aqueous solutions. Conglutinin or X protein seems to be a colloidal complex of albumin, globulin and phospholipid into which it readily dissociates upon dilution. Oxalated plasma, obtained by collecting blood in tubes containing *dried* oxalate powder, is a much better source of conglutinin than blood serum or purified serum albumin. This suggests that fibrinogen is another important constituent of X protein.

†The present author has recently succeeded in producing high-titered agglutinating anti-Rh sera in *normal, male* blood donors.

of the microscope. The reactions can often be improved by centrifuging the tubes for 1 minute at low speed, or by allowing the mixture to stand another hour or two at room temperature.

Some of the more important sources of error are as follows:

1. Poor blood samples are the most frequent cause of difficulty in performing the test.

A. Whole blood keeps well for periods up to 5 to 7 days in the refrigerator as a clot or mixed with 1/5 volume of 3.8 per cent sodium citrate solution. But the blood suspensions must be fresh. In warm weather blood suspensions may deteriorate within a few hours.

B. Hemolyzed blood samples give poor reactions. This can result from wet syringes, contamination of blood with alcohol and other chemicals, heating, freezing, etc.

C. Small samples from the finger, mixed with tissue juices, give poor results.

D. Infected blood samples give weak reactions or false (bacteriogenic) agglutination.

E. When in doubt obtain a fresh blood sample from the vein.

F. Blood suspensions are worthless for mailing purposes. Always send whole clotted blood.

2. Blood suspension should be 2 per cent strength in terms of blood sediment. Too weak (e.g., 1/2 percent) or too strong (e.g., 10 percent) suspensions give poor results.

3. The tube should be about 7 mm. to 8 mm. wide. Very wide tubes favor evaporation and the reactions are not distinct. Too narrow tubes are difficult to manage.

4. The water bath should be set at 37.5° C. Though the reactions will occur at room temperature, they are usually weaker at lower than body temperature. On the other hand, too high temperatures damage cells and serum.

5. Too rough handling breaks up the reaction. Just roll or twist the tube to dislodge the sediment for microscopic reading. Do

not shake. To avoid unnecessary handling, read reactions in the original tube and do not transfer to a slide.

6. Do not mistake swirls of cells and clots for agglutination. In true positive reactions, the cells are strongly clumped with no or only a few free cells between the clumps.

7. Use saline and not citrate solution for preparing the blood suspension.

8. Tubes must be scrupulously clean. Soapy, dirty or old etched tubes give poor results.

9. Tubes should have round or conical bottoms, not flat.

10. Contaminated and out-dated sera may give false positive or negative reactions.

11. At least one known Rh-positive blood and one Rh-negative blood should be included as controls with every test.

Technique for Rh Testing Using Conglutinating Sera Only[3, 4]

Mix one drop of the reagent with a drop (each drop .05 cc.) of fresh blood suspension of 2 percent strength in terms of blood sediment, in a small narrow test tube (7 to 8 mm. inside diameter). *(All blood suspensions must be washed once with saline solution.)* Incubate the mixture for 30 minutes in the water-bath at body temperature, and then centrifuge the tubes for one minute at low speed (about 500 r.p.m.). With a fine capillary pipette remove the supernatant fluid *as completely as possible* and discard.* Then add one drop of group AB plasma (or serum) and resuspend the sediment by strongly shaking the tube. Re-incubate in the water-bath for another 45 to 60 minutes, or until sedimentation is complete. (This reading is sometimes not as clear as in the agglutination technique, particularly if plasma instead of serum is used as the suspending medium. However, this is of no consequence as the final readings depend upon the microscopic appearance.) The sediment is dis-

*This is one of the most important steps in the technique. If any liquid is left behind, this will dilute the group AB plasma and weaken its conglutinating action.

lodged *by gently tilting or twisting the tube, and the reactions are read without removing the mixture from the tube* by placing the tube under the low power objective on the stage of the microscope. As a rule, there will be no difficulty in reading the reactions because in positive tests the clumps are large enough to be easily visible to the naked eye, while in negative reactions, the red cells separate from one another producing a uniform suspension as viewed under the microscope. In rare cases of doubt, the tubes may be allowed to stand another hour or two at room temperature and the reactions reread; or better, the test should be repeated.

The pitfalls in the conglutination technique are largely the same as for the agglutination technique. (See sources of error under Technique of Rh Testing with Agglutinating Sera). If group AB plasma is not available, use the patient's own plasma or any compatible oxalated plasma.

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AN EXPERIMENTAL EVALUATION OF THE MAZZINI SLIDE TEST*

By MARGARET M. DAVIS

From the Serologic Division, Bureau of Laboratories, Texas State Department of Health.

A growing interest in the Mazzini Flocculation Test for the serodiagnosis of syphilis has developed since the publication of the technic (1939) and the demonstration of the high sensitivity and specificity rating of the test in the Washington Serology Conference (1942).

Dr. A. W. Ratcliff (1940) made an analysis of 502 case studies (by the questionnaire method) collected in preliminary attempts to evaluate the Mazzini flocculation test for syphilis (1939). The data he presents indicates that the Mazzini is more sensitive than the tests with which it is compared and is interpreted as indicative of a satisfactory specificity. He points out that within the so-called positive range of three plus and four plus, the specificity is expected to be much greater. Ratcliff concludes that the Mazzini Flocculation test offers a greater aid in the serodiagnosis of syphilis than do other tests in common use. He advocates the use of multiple tests as preferable to any single test.

Ratcliff and Martin (1940) have made a study of the prezone phenomena in various flocculation technics for the serodiagnosis of syphilis. By quantitative titration of sera suspected of prezone phenomena, a predisposition to this masking reaction occurs in the Kahn Standard, Kline Diagnostic and Mazzini flocculation technics in the order named. The observation is made that the predisposition to prezone occurs in inverse proportion to the sensitivity of these tests.

In October, 1942, the Serologic Division of the Bureau of Laboratories, Texas State Department of Health began an investigation

*Award paper (Third), June, 1945.

of the production of Mazzini Flocculation Antigen, and a comparative study of the serodiagnostic results obtained from 1738 sera.

The blood sera studied were from the following sources: 1195 from Selective Service Boards, 64 from Blood Bank donors, 479 from Local Health Officers and Directors of Local Health Units. Of these sera 199 were unsatisfactory for examination so final results are based on studies of 1539 sera.

Procedure

All antigens used were produced by the Serologic Division and approved by the Author Serologists.

Mazzini Slide Test Antigen was prepared according to directions (1939). The titration and standardization is simply and rapidly done. Since it is impossible to obtain extracts of uniform antigenic value, the optimum lipoid-cholesterin ratio is determined by titration. Once the desired sensitivity is obtained, the routine technic for the preparation of the antigen suspension is easily performed

The Kline Exclusion Test and the Mazzini Slide Test were run simultaneously on all sera. On sera giving positive or doubtful Kline Exclusion or Mazzini results, the Kahn Standard Diagnostic and the Kolmer Complement Fixation were performed.

The Mazzini test was studied from the standpoint of comparative reactivity only. No attempt was made to evaluate the sensitivity and specificity as clinical data were not available on the sera used.

Results

Of the 1539 sera tested, 1172 were negative in both Kline and Mazzini; and 141 were four plus in Kline, Mazzini, Kahn and Kolmer (85.31 percent in agreement); in 145 tests Kline reactivity was greater than Mazzini (9.42 percent); in 12 tests Mazzini reactivity was greater than Kline (0.77 percent); in 69 tests there was agreement in Kline and Mazzini with disagreement in Kahn and Kolmer (4.5 percent).

Table 1 summarizes reactivity with the 367 Kline and Mazzini positive or doubtful sera. It will be noted that the Mazzini is more reactive than the Kahn and Kolmer, although less reactive than the very sensitive Kline Exclusion.

TABLE 1

Test	Positive	Doubtful	Negative
Mazzini Slide Test	268	47	52
Kline Exclusion	318	48	1
Kahn Diagnostic	224	15	128
Kolmer Complement Fixation	242	0	125

Discussion

From studies of the production and performance of the Mazzini Slide Test Antigen, it is obvious that the test has advantages technically. The buffered saline employed in the antigen suspension eliminates the frequent checking of the pH which is necessary in other test suspensions. The antigen emulsion is easily and rapidly prepared. Mr. Mazzini has made suggestions which will aid in the production of large quantities of the Flocculation Antigen.

The Mazzini Slide Test appears to give better than average sensitivity as performed by the Author Serologists, the U.S.V.D. Research Laboratories, and the Serologic Division of the Texas State Department of Health in Interstate and Intrastate Evaluation Studies for 1943 and 1944. An average increase in sensitivity of the tests analyzed from 1943 to 1944 was 8.63 percent as performed by the Author Serologists in the Interstate Evaluation Studies. The increase in sensitivity in the Mazzini Test was 6.2 percent as performed by Mr. Mazzini.

Summary

1. An experimental study of the production, titrations, and standardizations of the Mazzini Slide Test antigen by the Serologic Division of the Texas State Department of Health gave the following results:

a. The antigen can be satisfactorily produced in large scale by the Division.
b. The sensitivity of the antigen can be determined by simple titrations and standardization.
c. The test can be easily and rapidly performed.

2. A preliminary comparative study of the reactivity of the Mazzini Slide Test antigen and other serodiagnostic antigens gave the following results.

a. There was complete agreement of all tests in 1313 or 85.31 percent of the sera examined.

b. Kline Exclusion test reactivity was greater than Mazzini in 145 sera or 9.42 percent of the tests.

c. Mazzini test reactivity was greater than Kline Exclusion in 12 sera or 0.77 percent of the tests.

d. There was agreement of the Kline Exclusion and Mazzini tests with disagreement in the Kahn Diagnostic and Kolmer Complement Fixation tests in 69 sera or 4.5 percent of the tests.

3. The Mazzini Slide Test appears to give better than average sensitivity.

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EXPERIENCES OF A MEDICAL TECHNOLOGIST IN THE CANAL ZONE*

MARIANA D. NICHOLAS
B. S., M. T.

A few months after the war was declared, a skeleton crew was sent out to open up a new hospital in the Canal Zone. It was situated back in the jungles in contrast to the other hospital on the Atlantic side which was on the beach and directly in sight of the breakwater on the mouth of the Canal. There were four nurses, two doctors, two clerks, a pharmacist, a dietician, a technologist, and a group of silver men who were to act as orderlies. The hospital, which had originally been planned as a unit to care for the employees of the new Third Locks construction in the Canal, was only partly finished. It was built on a hill on such recently cleared land that the jungle grew right up to the doors. It was of typical tropical construction—grey clapboard, with tin roofing. The entire building was built up in the air on cement pillars to protect it from moisture and insects. Truly, the building was only a sprawling group of screened halls since it was all one story and the halls were screen from the ceiling almost to the floor. Rooms were indicated by walls rising part way to the ceiling. There were no windows and the only protection from the rain were the wide overhanging eaves. Most of the time this was sufficient but when the windy rains came often they blew right through the building.

Transportation was difficult since there was no available living quarters at first and personnel were carried to and from on ambulances. Likewise we only had a little equipment and the first months were spent ordering and installing our equipment. Only one ward out of the eventual seven we were to have was opened. We had

*June, 1945

ten convalescent patients that first day—transfers from the much overcrowded hospital on the beach. So much of the place was under construction that we had trouble at first with the jungle creatures which would wander in in the night. Once a queer shuffling noise kept half the patients and the night nurse scared almost to death most of the night. It turned out to be a little lost coon which could not find his way out of the maize where he had wandered. Once we pursued a little honey bear for two days before we finally caught him. And sometimes the creatures which found their way into the hospital were not so harmless. About a week after we opened, I opened the laboratory door one morning to find a coral snake coiled up just inside!

Coupled with the difficulties of opening a new hospital with only a minimum of supplies, we had all the difficulties of a community on a wartime basis to cope with. The day war was declared, the Canal Zone went on strict alert. Blackout was enforced as strictly as in any war zone, and there were no lights permitted from sundown to sunrise. At night, hospital work was carried on with the old cowbarn lanterns shielded with paper sacks. These were used only under extreme conditions and in completely blacked-out areas. The blackout often caused great hardship on the doctors and the nursing staff. One section of the laboratory and one operating room were equipped with heavy blackout curtains, which shut out all the light from the outside, but allowed us to work when necessary. In case the Zone was alerted all electricity was cut off at a central point and there was no choice except lantern light. It is difficult to imagine the difficulties such a situation causes a hospital but most of them can be surmounted if necessary.

There is very little difference in laboratory work in the tropics and in any other place in the world. For me the hardest part of work in the Canal Zone was parasitology. Approximately ninety per cent of the natives are infested with hookworm, ascarids or whipworm; or a combination of all three. Consequently feces examinations for parasites and ova were routine. Malaria, too, being so common, was a routine procedure in the laboratory. Every patient with a temperature of 100°F. or over had routine smears for malaria. I learned to take and strain smears for B. leprae from

nasal lesions. I saw my first clinical cases of yellow fever and filariasis. Otherwise, we ran a laboratory exactly as they are run the world over.

After we had opened the hospital more fully, we began receiving war casualties. Mostly these were the victims of torpedoeings in the Carribean. Sometimes they were horribly burned and often they had been at sea for days at the mercy of the sun and water. When boatloads of these patients were brought in, we all worked long and hard until they were admitted and given emergency care. Once we had a whole group of patients who had received yellow fever vaccine that caused a jaundice. I did icteric indices and van den Bergh's until I never wanted to do another one.

Worst of all, I think, though were the alerts. Every time that the Zone was alerted everyone had to report to duty as soon as he could. I lived two miles from the hospital and mostly the alerts were at night. That meant a two-mile walk up through a lonely jungle path. At the time there was no road to the hospital and the path was bordered on one side by swamp and the other side by jungle. It was a weird experience and sometimes frightening, to walk up through there in the dead of night. And after reporting at the hospital, we just waited in the dark, with no lights other than lanterns. If we worked, it was in the light of lanterns and flashlights, and it can be quite an undertaking to do laboratory work under such conditions. However, it wasn't too bad, because such work usually consisted of typing for transfusions or emergency white blood cell counts. One of the most annoying parts was the lack of adequate supplies and the difficulty of getting them. A great many things had to be ordered from the States and even then were often lost on the way down.

One of the things that impressed me most in the Canal Zone was the response that we had to our appeal for blood donors. The day after war was declared the Zone sent out by radio and newspaper an appeal to all civilians to report to the hospital for blood typing and Wassermanns. This was to be done in case we had large scale casualties to treat we would have an available list of volunteer donors. To my utter amazement the number of natives who came in and offered their blood to help others was as great as the white

people. In fact, the first man in line was an old Negro bartender from Colon with one arm, who said that if his blood could help any one who needed it, they were more than welcome to it. The response among such people gave us a warm feeling toward the human race in general, for though very few of them could have been used, at least their hearts were in their desire to help.

In the Canal Zone, all personnel are divided into two groups—Silver and Gold. This dates back to the early days of the Isthmian Canal Commission when the native labor was paid in silver and the white labor was paid in gold. We had large numbers of silver patients in both the hospital and dispensaries. These people often spoke only Spanish and sometimes that was difficult for the technologist, especially when doing gastrics or other things which required explanation and cooperation on the part of the patient. Likewise, technologists in the Zone are to a certain extent spoiled because they usually have a fairly large group of silver employees who do some of the work that is done by the girls themselves in other places—such as urinalyses, making solutions, etc. Sometimes, keeping peace among these employees is such a job that the technologists wish they didn't have to be bothered with silver help—at least I did. It takes a combination strong arm man, diplomat and bully to do it sometimes.

We had an old silver woman who came to the clinic to be treated for some sort of undiagnosed anemia one time. She came in repeatedly for blood counts and she thought that the doctors and the hospital were wonderful. She said that she felt so much better when she came in, and she was such a good patient that we all liked to see her. However, she was an old native from out in the jungle and she had not by any means given up her "jungle medicine". One day I came to work with a terrible case of hives. My legs and arms were swollen and covered with itchy weeping blotches. Old Sara came in that morning for a blood count and gazed for a long time at my hives. Finally she asked me if I was uncomfortable and if I wanted to get rid of the spots. Then she proceeded to give me a "sure cure". It consisted of catching a speckled frog in the jungles after sundown. After I caught it, I was to rub it all over the spots on my arms and legs, and then kill it. She said that if I

wore it around my neck all the spots would go away and never would I have them again. In fact she even offered to go catch the frog for me. My first contact with the "medicine of the jungle" which is a mixture of superstition and ritual! She was one of the most interesting patients that ever came into the laboratory.

In the years that I have worked in the Zone, I think that the part of the work I liked the most was parasitology, especially malaria. I found all of Panama interesting and different, but the hospital system was probably like almost any army hospital in the world. The big job of keeping the Canal Zone sanitated and as free of disease as possible is done, not so much by the hospital as by the sanitary corps men who work out in the jungles and swamps. Only after one has been up into the interior where mosquitoes, food and water control are at a minimum can one realize the big job that is done outside the hospital and the laboratory proper.

PERNICIOUS ANEMIA*

By NOBUKO S. MIZUNO

Possible Approach to Early Laboratory Diagnosis

Pernicious anemia is a condition first described by Addison in 1849 and 1855 (1) and by Biermer in 1872 and is sometimes referred to as Addisonian anemia or Biermer's anemia. It is a disease of unknown etiology characterized by macrocytic anemia associated with disturbances of gastro-intestinal tract and frequently of the nervous system. The clinical aspects of the disease are discussed briefly under the following headings:

I Distribution
II Physical Findings
III Blood Findings
1V Urine and Stool
V Gastric Analysis

I Distribution

Pernicious anemia occurs mainly in the temperate climate, chiefly in the blue-eyed or Nordic types and is more prevalent in North Europe, North America and the British Isles (2).

Age: It usually occurs in late adult life or old age. The average group was 40-60 years. It rarely occurs in persons under 30, and does not occur in children.

Sex: More cases of pernicious anemia are found to occur in men than women.

Familial predisposition: McLachlan and Kline (3) report of the occurrence of anemia in four generations. There seems to exist a hereditary predisposition towards achlorhydria.

From Clinical Laboratory of the Chas. T. Miller Hospital. Presented at Weekly Laboratory Staff Seminar.

II Physical Findings

Onset: The onset of the disease is gradual and insidious and symptoms of anemia per se are seldom obvious until hemoglobin has fallen below 65 per cent. The disease may occur with mild relapses in early stages or with intermittent attacks of symptoms.

Initial symptoms: The initial symptoms are variable. Gastro-intestinal symptoms or nervous manifestations may be present before any clearcut evidence of macrocytic anemia is seen.

According to Musser and Wintrobe (4) the common initial symptoms are in order of frequency: fatiguability, weakness and faintness, numbness, tingling and stiffness, headache, nausea, lack of appetite, vomiting, dizziness, dyspnea, palpitation, diarrhea, loss of weight, pallor, abdominal pain and sore tongue.

Isaacs (5) studied 1000 cases of pernicious anemia and found the following symptoms with their degree of frequency:

ease of fatigue and generalized weakness	85%
numbness	80
tingling finger and toes	74
shortness of breath	64
symptoms referable to stomach	62
constipation	64
palpitation	50
edema	44
loss of appetite	44
difficulty in walking	44
symptoms referable to bladder	35
dizziness	28

Symptoms of the gastro-intestinal tract: Recurrent, burning and soreness of tongue is frequently found. These usually involve the anterior half of the tongue and is especially pronounced on ingestion of hot or spiced foods. The tongue may be swollen and indentation of teeth can be seen. It may be red with evidence of ulceration or formation of small blisters. Later the wasting of papillae with vesicle formation leads to a smooth, shiny, atrophic tongue characteristic of the disease. A similar process may extend into the esophagus and the stomach.

True achlorhydria or achylia is a feature of the disease. There is loss of appetite, nausea, vomiting, feeling of fulness, belching, passing of gas with foul-smelling stools, intermittent periods of constipation and diarrhea.

Neurologic disturbances: In one-fourth of the patients, these are the first to appear. In majority of the patients, the symptoms of neurologic involvement are confined to slight disorders of sensation, persistent and usually symmetrical numbness and tingling of the hands and feet. Among the earliest of the objective signs is a diminution of vibration sense at ankles, usually but not always symmetrical. Development of marked spastic ataxia indicates degeneration of the lateral and posterior columns of spinal cord. When former tracts are particularly involved, spastic gait, increased reflexes and positive Babinski's signs are usually present; with lesions of latter tracts, loss of vibration and position sense and ataxia appear. Girdle sensation occurs, but lightning pains, girdle pains and abdominal crisis are uncommon. Due to peripheral nerve involvement, "stocking" hypoesthesia (decreased sensation) or anesthesia may occur. Occasionally hyperesthesia (increased sensation) of soles and feet may be very marked. Disturbances of heat and cold is unusual.

Circulatory disturbances: These are dependent on the severity of the anemia. They may include dizziness, tinnitus, palpitation, some dyspnea, excessive weakness and fatigue. Heart rate is usually increased, but no murmurs or irregularities are heard. In general, circulatory changes are identical with those present in any type of severe anemia.

III Blood Findings (7)

When cases are seen early or in remission, the changes in blood may be slight or absent. When the patient is in relapse, a typical blood picture may be easily recognized. These include changes in the following:

Erythrocyte: The red blood cell count is usually less than 2,500,000 per cu. mm. The erythrocytes show practically any of the abnormalities known to occur except pronounced achromia. The characteristic feature is a large percentage of macrocytes. Other abnormalities include:

microcytosis
anisocytosis
poikilocytosis
diffuse or punctate basophilia
reticulocytes above 5% is uncommon
variety of nucleated red blood cells may be seen, including megaloblasts

Cabot ring bodies and Howell-Jolly bodies
mean corpuscular volume large, usually 110-160 cu. micra
mean corpuscular hemoglobin 32% or more
color index above 1 almost always, often 1.5 or more

Leukocytes: These changes are not characteristic. In relapse, there may be variable degree of leukopenia (2,000) with granulocytes reduced in number, resulting in relative but not an absolute lymphocytosis.

There may be a shift to the right since the granulocytes exhibit hypersegmentation of nuclei. This is not a consistent finding. There may be a shift to the left of the leukocytes in patients with extremely low white blood counts.

There may occur eosinophilia in patients under treatment with raw calf's liver.

Platelets: Although the platelets are generally diminished, they very rarely fall below 40,000 per cu. mm. The decrease is roughly proportional to the decreased red blood count.

There may be increased bleeding time, delayed clot retraction time, but the coagulation time is normal.

Bile pigments: There may be increased icterus index (up to 20) and increased plasma bilirubin. The indirect van den Bergh is positive.

Bone marrow: In pernicious anemia and related macrocytic anemia, a study of bone marrow shows degree of primitive cell proliferation which is in direct proportion to the severity of the anemia (5). The primitive cells are megaloblasts, similar to, but not identical with, the primitive erythroblasts of the embryo. They are not present in either normal bone marrow or hyperplastic marrow due to chronic hemoragic or hemolytic process (8). In spite of the continued need for red blood cells in the body, maturation of

the megaloblasts into erythroblasts and adult erythrocytes is apparently arrested until the specific hematopoietic principle is supplied. When this is done, then there is a rapid maturation of the primitive cells through the normoblastic stage into the reticulocytes and mature erythrocytes. This is due to the supplying of the specific deficiency and not to a general stimulus to maturation as suggested by the experiment that liver extract did not interfere with the rate of division of the red blood cells in the chick blastoderm (9).

IV Urine and Stool

Urine: Urinanalysis may indicate chronic nephritis with fixation of specific gravity, small amount of albumin, variable number of hyaline and granular casts and low urea clearance. There is an increased excretion of urobilin and urobilinogen due to increased biliary metabolism. It does not contain any appreciable amount of the hematopoietic principle (10). There may be an increased excretion of coprophoryrin I (11), the rate of excretion of which is an index of the bone marrow activity.

Stool: There is no characteristic stool, as one may find in sprue. It is usually darker than normal due to increased biliary pigment.

V Gastric Analysis

Characteristically there is true achlyia, as checked with histamine. The achylia is found at all times, in relapse and remission. This is the single pathological finding which seems unaffected when all other evidences of the disease disappear with proper treatment.

Factors Involved in Pernicious Anemia

Results of Whipple and Robscheit-Robbins (12) in their studies on anemic dogs showed that liver and kidney were especially effective in the regeneration of hemoglobin. Minot and Murphy (13) acting on this suggestion, found that everyone of the 45 cases of pernicious anemia patients improved almost at once under liver treatment therapy and continued in good health as long as the liver was regularly eaten in sufficient quantities. Castle and Locke (14) found that the administration of liver or liver concentrate alleviated all the major symptoms of the disease except one, namely, the inability of the stomach to secrete hydrochloric acid and enzymes which normally produce hydrolytic cleavage of the proteins of the food in gastric digestion. Castle (15, 16) treated pernicious anemic

patients with gastric secretions of normal individuals and found that it was ineffective in bringing about blood regeneration. Daily feeding of 200 gms. of beef muscle had no significant effect. When the patients were given raw ground muscle meat which had been predigested in the stomach of a normal individual, all the improvements characteristic of liver therapy were observed. This led Castle to postulate that the gastric secretion of the normal individual contains an "intrinsic" factor which acts on an "extrinsic" factor in association with proteins of some foods to develop the hematopoietic factor necessary for the maturation of the red cells. The hematopoietic factor was not present in either meat or normal gastric juice, but was the product of the interaction of these substances during digestion. Clearly then, there are at least three factors involved in pernicious anemia. They are:

(1) intrinsic factor (normal gastric juice)
(2) extrinsic factor (beef muscle)
(3) hematopoietic factor (liver, or normal gastric juice plus beef muscle)

Nature of the intrinsic factor:

Site of secretion: Meulengracht (17, 18) found the activity of the pig stomach to be largely confined in the pyloric portion and the active material was also found in the upper portion of the duodenum, where glands similar in structure to those of the pyloric region are present. Dexter et al (19) found that if thoroughly washed, the lower half of the small intestine is inert suggesting that any activity from this portion of the bowel results from the absorption of the intrinsic factor which have been secreted by the stomach and the duodenum. However, it is not to be assumed that any observation on the hog can be said to necessarily apply to man.

Fox and Castle (20) found that in human stomach those areas containing the fundus type of gland and not the pyloric gland organ had the highest anti-pernicious anemia activity. Meulengracht (21) also found that in pernicious anemia the stomach showed atrophy of the chief and parietal cells in the fundus portion, but in the pyloric portion and the duodenum, normal glandular structures were preserved, thus the source of the intrinsic factor appears to coincide with the site of degenerative processes seen in the histologic preparations of stomach in pernicious anemia.

Nature of the intrinsic factor: The effective agent in the stomach is thermolabile and is rapidly destroyed by temperature above 70° C and more slowly by temperature as low as 40° C. It has not been identified with *any known constituent* of normal gastric secretion. It is not hydrochloric acid, pepsin, rennin or lipase. It is not present in normal human saliva or normal duodenum secretion. It may be present in patients with anemia other than pernicious anemia and it may be present in certain unusual cases of macrocytic anemia in presence of normal amounts of hydrochloric acid and of pepsin and rennin.

Taylor et al (22, 23) observed that normal gastric juice contains a proteolytic enzyme which is capable of hydrolyzing casein to the proteose stage in an alkaline medium. This enzyme was differentiated from pepsin by virtue of its inactivity at hydrogen ion concentration below 4.0 and from trypsin in that it does not produce a large amount of amino nitrogen within 24 hours.

Certain similarities exist between the properties of this proteolytic enzyme and the intrinsic factor. These may be summarized as follows:

(1) Both can be removed from gastric juice by absorption with Lloyd's reagent at pH of 7.4.

(2) Both are impermeable to certain semi-permeable membranes.

(3) The activity of the enzyme was retained and destroyed under conditions of temperature and acidity which retain and destroy the intrinsic factor.

(4) Such proteolytic enzyme activity was found minimal or absent in patients with pernicious anemia (24).

Nature of the extrinsic factor:

Source of the extrinsic factor: The extrinsic factor is found in beef muscle, egg white, milk, casein, tomato, spleen autolyzed and autoclaved yeast, rice polishings and wheat germ. It differs chemically from the hematopoietic factor because hydrolysis of liver extract with sulfuric acid until its active principle for pernicious anemia has been destroyed, did not destroy the extrinsic factor. It can be differentiated from the hematopoietic factor by virtue of the fact that a substance having the hematopoietic factor will be effec-

tive when given intravenously or intraparentally, but a substance containing the extrinsic factor alone must be acted upon by the intrinsic factor before it can be effective by such routes.

Nature of the extrinsic factor: The extrinsic factor is thermostable. Its close association in nature with foods rich in the vitamin B complex (yeast, wheat germ, rice polishings) suggests that it may be one of the members of the vitamin B complex, although to date it has not been possible to identify it with any of the known components of the vitamin B complex (25).

Recently there was much interest aroused by the discovery and the isolation of vitamin B_c, which was effective in curing macrocytic anemia in chicks. Hogan (26) was able to produce macrocytic hyperchromic anemia in chicks with dietary deficiency which could be cured by liver, but not by any known vitamin (27, 28). Pfiffner et al (29) was able to isolate in crystalline form, vitamin B_c, which cured macrocytic anemia in chicks. Sharpe et al (30) gave 1,000 to 1,500 gamma of vitamin B_c in form of yeast to anemic patients refractory to treatment but found little change in the erythropoietic phenomenon. There is as yet no clinical data on the use of vitamin B_c in pernicious anemia patients. In conclusion one might say that the extrinsic factor appears to be one of the thermostable components of the vitamin B comples which is yet to be identified.

Condition necessary for the interaction of the intrinsic and the extrinsic factors:

Patients with pernicious anemia responded when substances containing the intrinsic and the extrinsic factors were given as much as 6 hours apart. No effect was obtained unless the mixture was brought to a pH between 5 and 7. Incubation in vitro was not essential to produce the reaction. Heating to 70-80 degrees C for ½ hour or boiling for 5 mins. destroyed the activity of the mixture of the intrinsic and the extrinsic factors even after incubation with gastric and duodenal contents; therefore, it was not possible to find in the incubated mixture of the intrinsic and extrinsic factors, any factor identical with the active principle (31).

It has been claimed that the interaction of the intrinsic and the extrinsic factors follows mass action law and can be represented symbolically by the following:

$$\frac{F \times G}{I} = H$$

F = food or extrinsic factor
G = gastric or intrinsic factor
I = intestinal permeability
H = hematopoietic factor

Any or all of the changes from the normal (F, G, I) will create a decrease in the body of the hematopoietic factor. Certain quantitative relationship exists between F and G, so that an excess of one may compensate for the deficiency of the other. Therefore if an excess amount of the extrinsic factor is given to a patient with greatly diminished but not completely absent intrinsic factor, enough hematopoietic substance might be formed to alleviate the anemic condition. This might be the underlying explanation for the conflicting reports of the effectiveness of yeast (which is considered to be a source of extrinsic factor and not the hematopoietic factor) in curing pernicious anemia (32, 33).

The hemotopoietic factor:

Throughout the discussion the use of the term "anti-pernicious anemia" factor was avoided, and in its stead the term "hematopoietic" factor is used. This was to avoid certain erroneous fixations in the mind that the lack of this factor is associated with the condition of pernicious anemia alone; which is not true, as will be seen in the next topic; or, that the lack of this factor alone is the cause of pernicious anemia; which is again not true, for the lack of the intrinsic factor appears to be a more basic feature of the disease. The term "liver factor" or "active liver principle" is also misleading for this factor is found not only in the liver, but in the kidney, brain, placenta and other organs.

The effective agent in the liver is thermostable and is resistant to boiling temperature. There is much disagreement as to the properties of the hematopoietic factor, probably due to the failure in isolating it in pure state. As a matter of fact, some have upheld the multiple nature of the liver factor (34). However, as long as the liver principle remains in an impure state, every therapeutic preparation is a mixture of several different substances, one or more of which is the effective agent. Dakin (35) made attempts to purify and identify the hematopoietic factor and believed that it was a substance associated with a peptid and possessed many but by no

means all the properties of an albuminose. The peptid on hydrolysis yielded arginine, leucine, glycine, prolie, hydroxyproline, aspartic acid and an acid resembling hydroxyglutamic acid. The molecular weight is probably between 2000 and 5000. Karrer (36) isolated hematopoietic factor which was active in single doses of 0.8 to 0.15 mgm. The highly purified principle was precipitated by ammonium sulphate, was non-homogenous, and gave weakly positive biuret and positive ninhydrin reaction. The probable molecular weights were 3,000, 6,500 and 14-15,000. It was not a common polypeptid, probably a compound of a polypeptid. A later analysis (37) revealed the presence of the amino acids: histidine, arginine, lysine, glutamic acid, leucine, alanine and valine—all of which account for 15 per cent of the compound.

The hematopoietic factor is found in the liver of practically all mammals (38). Little or none was found in the liver of patients with classical Addisonian anemia or with macrocytic anemia of sprue who died without specific treatment.

Theories as to the causation of pernicious anemia:

The present concept is that the conditions necessary for the normal maturation of the erythrocytes consist of the following requirements:

(1) presence of extrinsic factor in food.
(2) elaboration of intrinsic factor by the glands in the stomach.
(3) interaction between the two in the small intestine to produce the hematopoietic factor.
(4) absorption of the hematopoietic factor from the small intestine.
(5) transportation of the factor by blood to liver, bone marrow and other organs.
(6) storage of the factor for further use particularly in the liver.

Any interference with the above chain of events result in the deficiency of the hematopoietic factor in the body and result in macrocytic anemia. This is supported by the fact that macrocytic anemia is associated with many conditions other than pernicious anemia, and can be traced to lack in the diet, faulty absorption or utilization of one or more of the essential factors. Macrocytic

anemia may occur in the following (discussion taken largely from Kracke's "Diseases of the Blood"):

(1) anemia of sprue.
(2) pellagra
(3) extreme liver damage.
(4) leukemic states.
(5) certain anemias of pregnancy.
(6) idiopathic steatorrhea.
(7) intestinal disfunction, chronic diarrheas.
(8) intestinal anastomoses.
(9) advanced cancer of the stomach.
(10) diphyllobothrium latum infection.
(11) many anemias of childhood.
(12) anemia of alcohol addicts.

Macrocytic anemia which occurs in sprue and pellagra can be traced to the lack of extrinsic factor in the diet. Achlorhydria, if present, can be corrected after adequate treatment. Yeast is as effective as liver in the cure, suggesting that the lack of extrinsic factor is the probable cause.

Macrocytic anemia which occurs in certain cases of pregnancy may be traced to one of the following: (1) deficiency of extrinsic factor in diet, (2) failure of interaction of the intrinsic and extrinsic factors in stomach due to pernicious vomiting and nausea, (3) failure of absorption of the hematopoietic factor or (4) incapacity of liver for storage due to toxemia. Usually there is some HCl in the stomach after histamine. Either yeast or liver extract is effective in the remission.

Macrocytic anemia may occur in long standing liver disease or far advanced destruction of liver tissue followed by fibrosis, as in nodular atrophic cirrhosis and Banti's disease. In these conditions the liver may be so altered that it is incapacitated in its function to store, synthesize or release the hematopoietic material and the anemia results. Macrocytic anemia is known to occur in cases of alcoholism (38, 40). Factors which may operate to produce anemia by gastric disfunction, dietary deficiency or hepatic damage, causing the failure of liver to store the hematopoietic factor.

Pernicious anemia may result from gastrectomy and gastroenterostomy and advanced cancer of the stomach. Several factors may influence the development of the macrocytic anemia after resection of the stomach:

(1) extent of impairment of gastric and intestinal function.
(2) amount of reserve hematopoietic factor stored in the liver.
(3) age of patient and presense of infection.

Macrocytic anemia may result from interference in absorption. Experimentally it was possible to produce in dogs by internal bile fistula, a condition of macrocytic anemia which was curable by purified liver extract parentally (41). Clinically it is manifested in:

(1) prolonged diarrheas of amebic and bacillary dysentry.
(2) ulcerative colitis.
(3) intestinal strictures.
(4) idiopathic steatorrhea.
(5) infestation with fish tapeworm.

It appears that macrocytic anemia can be traced to any interference with the chain of processes which makes the hematopoietic factor available to the body.

The peculiar deficiency in pernicious anemia which results in macrocytic anemia appears to be the lack of the intrinsic factor. The achylia is found at all times in relapse and remission and is the single pathological finding which is not improved by treatment. The defect is permanent and irreversible. It seems unlikely that achylia results from anemia since it is known to precede the anemia sometimes by a matter of years; therefore, it is more probable that the cause of pernicious anemia is the lack of the intrinsic factor resulting from lesions in the gastric mucosa. Cox (42) made studies of stomach of patients who had pernicious amenia but had been receiving treatment for varying periods of time and found marked alteration in the mucosa of the fundus and body, in contrast to the relative freedom from abnormalities in the pyloric regions, confirming Meulengracht (21). The outstanding feature was the massive destruction of the highly differentiated chief and parietal cells. In the fundus region, the mucosa was only half the thickness of that of the pyloric zone, and it consisted of a completely abnormal type of mucosa in which the normal specific cell types (parietal and

chief cells) were absent. The mucosal glands were shorter, less numerous, more tortuous than those of the normal fundus region. Scattered through the abnormal mucosa were sometimes very numerous, coarse, deeply staining glands showing structural features characteristic of mucosal glands in the small intestine. He also studied stomach of a patient with sprue, who at the time of death had macrocytic anemia with presence of a large number of megaloblasts in the bone marrow. The stomach showed none of the changes described above.

Pernicious anemia have been known to result from gastroenterostomy (43). Goodman et al (44) studied the effect of gastrectomy on the liver of young swine and found that the effective hematopoietic factor was depleted from the liver.

In way of summary, following facts are repeated for the sake of emphasis: Achylia may precede macrocytic anemia sometimes by the matter of years. Achylia is the single pathological finding (excluding, of course, certain neurological changes which are known to be irreversible) which persists even after remission. Return of free HCl is rare. As a matter of fact, it is so unusual that the term "pernicious anemia" should be restricted only to cases characterized by anacidity. The existence of pernicious anemia should not be accepted without true achlorhydria (45). These facts are corroborated by histologic investigations which show permanent massive destruction of the glandular elements of the gastric mucosa. It is also shown that the removal of stomach can result in macrocytic anemia. All these facts point to the probability that the pathogenesis of macrocytic anemia in pernicious anemia is the deficiency of the intrinsic factor. What causes the destruction of the glands which results in the lack of the intrinsic factor is not known. Hereditary predisposition may play a contributing role.

Need for better aids for the diagnosis of pernicious anemia:

There is distinctly a need for better aids for the diagnosis of pernicious anemia. The onset of the disease is gradual and insiduous and the disease is usually well advanced before the physician is consulted. The average duration of symptoms was 1.36 years before the diagnosis was made (46). Symptoms of anemia per se are seldom obvious until the hemoglobin has fallen below 65 percent and even at this stage, differentiation from other types of anemia

is not always easy. To add to the difficulty, the patient may not show progressive development of symptoms since many of them take "shotgun" medication at irregular intervals so that the clinical picture is atypical. The disease may occur with mild relapses or with intercurrent attacks of symptoms.

The outstanding feature of the disease, which is macrocytic anemia is easily recognized, but does not appear until the disease is far-advanced. Cases which are seen early or in remission do not show changes in the blood picture. Furthermore, by the time macrocytic anemia has set in, there may already be neurological disturbances which may accompany the disease which are irreversible in character. Clearly then, it is necessary to seek changes in the pernicious anemia patients which may be detectable before macrocytic condition results.

From the preceding chapter, it appears that the pathogenesis of macrocytic anemia results from the lack of intrinsic factor; therefore, an analysis of this factor, if it were possible, would be an invaluable aid in the diagnosis. Although there are evidences that it might be a proteolytic enzyme, the exact nature has not been proved, and there are no methods for the estimation of this factor at present. Whether the diminution precedes, parallels or follows the diminution of hydrochloric acid is not known, but at any rate, the detection of free hydrochloric acid has been an easy and a convenient index of the functioning of the glandular secreting elements of the gastric mucosa.

The quantitative estimation of the extrinsic factor would be of little use, since the effective hematopoietic factor is derived from it, the amount depending on the adequacy of the intrinsic factor. The other object of study would be the hematopoietic factor.

In any known nutritional deficiency of a water-soluble nutritional factor, urinary excretion has been one of the earliest and best guides for the determination of the nutritional status. One can detect the decrease in urinary excretion of vitamins C, B and other vitamins before any physiological or histological changes and long before any blood changes are evident. One would therefore suppose that if this hematopoietic factor were excreted normally in the urine, the amount of which is influenced by the adequacy of the supply of the hematopoietic factor in the body, then one would sup-

pose that a urinary excretion of this factor would diminish when the supply of the hematopoietic factor to the body is diminished. Therefore, the first pertinent question to ask is: "Is there any evidence for the presence of the hematopoietic factor in normal human urine?"

There is very little work done pertaining to the presence of the hematopoietic factor in the urine. This is due in great part to the difficulty in separating without loss of potency the small quantities of the hematopoietic factor from large amounts of urine in a state suitable for parenteral injection, and free from toxic substances. Some of the work has been based on the reticulocyte response in rats, guinea pigs and pigeons (47, 48, 49). Reliable observers have shown that the results based on animal tests are not conclusive since similar reticulocytosis may arise spontaneously from change of diet, or under influence of substances present in the liver other than the hematopoietic principle. Reticulocytosis was even shown to fail to occur following administration of liver extract of know activity. Therefore the only reliable method is the clinical test of Wilkinson (50). Unfortunately there are few results by this method, but these seem to substantiate the presence of the hematopoietic factor in the urine (50, 51). Wilkinson's methods for the separation of the hematopoietic substance from the urine were as follows:

(1) Direct concentration: Samples of fresh urine were concentrated daily as soon as obtained to about 1/10th of their volumes at temperatures below 30° C in vacuo, using a Reich distilling apparatus. Equal volumes of 90 percent alcohol was then added, mixed and filtered. Filtrate was concentrated to 1/10th of its volume and 2 volumes of 90 percent alcohol were added. Resulting precipitate was filtered off and the filtrate was concentrated to a syrupy mass. It was poured into a large excess of absolute alcohol. The precipitate obtained was extracted with warm absolute alcohol to remove the urea and other impurities and was then washed with ether, dried in a vacuum desiccator and weighed.

(2) Norite absorption: The urine was treated in batches of 2 liters with 20 gms. of norite at pH of 5. After standing for 24 hours, the norite was removed by filtration and put into 400 ml of 60 percent alcohol. The daily samples, left at this stage until all

has been collected, were then mixed and boiled for 30 mins. After filtration, the norite was extracted twice with 1 liter of phenol, containing about 5 percent water, and the extract was treated with ether and water. The aqueous layer was mixed with 60 percent alcohol filtrate and concentrated in vacuo to a syrup. It was then poured into an excess of absolute alcohol. The resulting precipitate was washed with absolute alcohol, then ether, and dried in a vacuum desiccator.

The potency of the extracts were determined by hematological and clinical effects following intramuscular injection, after preliminary control periods, into well-authenticated pernicious anemia patients. Active extracts produced firstly a sharp rise or peak in the number of reticulocytes in the blood, and this was followed by an increase in the red blood cells and hemoglobin with simultaneous improvement in the clinical condition. By this method Wilkinson (50) and other workers (51, 52) were able to show that from large quantities of normal urine it was possible to prepare extracts which when given to untreated pernicious anemia patients will initiate remission.

Is the hematopoietic factor diminished or absent in the urine of pernicious anemia patients? Wilkinson (50) found that urine from pernicious anemia patients in relapse failed to furnish active extracts. When these patients were adequately treated and their blood and general condition returned to normal, then they passed urine from which active extracts could be prepared. Trager et al (53) found that urine of normal person or persons with aplastic anemia or leukemia contained a substance which enhanced the growth of mosquito larvae, Aedes Aegypti. This substance was found to be either lacking or present in much smaller amounts in extracts from urine of pernicious anemia patients. It was found in the urine of such patients after adequate liver treatment. Trager (54) later found that the mosquito larvae required pantothenic acid and pyridoxin for growth, so this method does not eliminate factors other than the hematopoietic factor.

Methods of detecting the hematopoietic factor in the urine: At present the only reliable method is the preparation of concentrates from urine suitable for intramuscular injection into well-established pernicious anemia patients. Although such a method

is of great importance in yielding results of theoretical importance, it is obviously impractical for clinical use. Tests of reticulocyte response in animal are of less specific nature and are still impractical for clinical use. Since several other factors beside the hematopoietic factor may enhance the growth of mosquito larva, Trager's method with the mosquito larva is of still less specific nature.

The method proposed here is a microbiological method with carefully controlled conditions. Microbiological methods have been used in the determination of some of the members of the vitamin B complex with success. As a matter of fact, the microbiological method for nicotinic acid with the use of Lactobacillus casei is at present one of the best methods for its estimation in biological fluids.

The principle of the microbiological methods is as follows: Microorganisms require certain nutritional factors for growth. Some of these essential factors have been identified as vitamins. This is an useful information since the rate of growth of microorganisms can then be related in some way to the amount of the nutritional factor. In order to work out a satisfactory method for the quantitative estimation of a particular substance, there are several things to consider:

(1) choice of the organism.
(2) substances which the organism requires for growth (must include the hematopoietic factor).
(3) determinations of optimal quantities of these substances for growth.
(4) method of measurement of growth: turbidity, acid production, etc.
(5) is growth proportional to growth? If so, the method is satisfactory.

The method consists of supplying in optimal quantities all the necessary factors to the organism except the one which is to be determined. The control receives known amounts of the factor which is to be determined, and similar preparations receive varying amounts of the substance to be tested for this factor. The growth of the organism of the unknown is compared in terms of turbidity or acid production or some other measureable property to the growth of the organism of the known or the control. The difficulty in the method lies in the determination of the optimum amounts of

the necessary factors and in determining what the essential factors are. The necessity for determining this is so that when the unknown in the form of blood, urine, etc., is added, the substances other than the factor in question which is added concomitantly should not add to the growth of that organism.

Possible value of determination of the hematopoietic factor in the urine: A method for the determination of the hematopoietic principle in the urine might serve as one of the earlier aids in the diagnosis of pernicious anemia. One might suspect that a decrease in its excretion might be detectable early in the course of the disease. However, since all conditions associated with macrocytic anemia can be traced to the lack or deficiency of the hematopoietic principle in the body, such a data would not differentiate the pathogenesis of the macrocytic anemia, but in conjunction with gastric analysis and clinical findings, it might serve as a useful diagnostic tool. Much work remains to be done before we can reach any conclusion with any degree of certainty.

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NEWS AND ANNOUNCEMENTS

All hotel reservations must be in the hands of the Housing committee by June 1st, as this is latest date that hotels will hold contracts for the A.S.M.T.

On to San Francisco! where we'll convene on June 25, 26, and 27, 1946. Has everyone mailed in an application for a room reservation to Miss Clara Becton, 78 Collins St.? Miss Becton is Chairman of the Local Arrangements and Housing Committee.

Miss Jeanne Jorgenson, 115 Frederick St., San Francisco 17, Cal., Chairman of the Entertainment Committee, has all sorts of interesting excursions planned for us. San Francisco is a convention city we all want to visit.

Be sure to meet with us! "There are many problems of vital interest to the medical technologist. We can accomplish much by discussing and working together. So, we'll see you in San Francisco."

ON TO SAN FRANCISCO, JUNE 24-28

The 1946 A.S.M.T. convention promises to be the most unique in the history of the society. With hotel accommodations scattered throughout downtown San Francisco, each person planning to come to the convention will have a much happier time if a party of two or more will arrange to occupy a room in a festive holiday spirit, especially since the O.P.A. has set forth definite rules regarding the price and number of occupants per room. Also, the double occupancy requirement for rooms should enhance the over-all attendance, if each A.S.M.T. will urge another A.S.M.T. friend to attend the convention.

An outstanding scientific and entertainment program has been planned. This year a large number of pathologists are giving many excellent papers to our group because we have the same hotel headquarters and overlapping convention dates.

The entertainment committee has spared neither time nor effort in arranging a topnotch program. Early registration will be provided on the evening of June 24, during an informal reception scheduled for the purpose of providing an opportunity for everyone to get

acquainted. City maps and descriptive posters on "points of interest" will be available for perusal during the reception, as a measure to give the out-of-town A.S.M.T. members a perspective of San Francisco and the Bay area.

San Francisco—America's Most Colorful City

Beautiful, gay and cosmopolitan San Francisco has rightly been called the "Paris of America." Its foreign quarters, picturesque in native dress, language, and customs . . . World-famous hotels, restaurants, and theaters . . . Metropolitan department stores, hundreds of smart shops, and strange bazaars . . . Impressive Civic Center flanked by the City Hall, Exposition Auditorium, Veterans' Memorial Building, and the most modern Opera House in the world . . . Historic Delores Mission . . . Quaint little cable cars plying up the steep San Francisco hills . . . Lofty Nob Hill, the sight of smart hostelries, palatial mansions, and Grace Cathedral (Episcopal) . . . Scenic Golden Gate Park and its unsurpassed museums . . . The Legion of Honor Art Galleries . . . The famous Cliff House . . . Fisherman's Wharf where hardy Mediterranean stock sing and shout in native tongue . . . Chinatown, the largest Chinese colony outside of China, where bazaars and stores cover blocks . . . The two great bridges, the Golden Gate and the San Francisco-Oakland Bay . . . Colorful Market Street with its four lanes of trolleys . . . The unsurpassed cosmopolitan night life . . . The Embarcadero, the terminal of 133 shipping lines . . . and many more interesting places contribute to making San Francisco the most colorful and romantic city of the West Coast and one of the most interesting in America.

San Francisco—A Must for Anyone's Holiday

Why not make the A.S.M.T. convention the start of your 1946 vacation? Plans have been made for a trip to the campus of the University of California, a four-hour tour of the City of San Francisco, a trek through Chinatown (occupying an entire evening), a dinner at the Balalaika Russian Restaurant, and a luncheon at the Hotel Claremont in Berkeley, in addition to the traditional annual banquet, which incidentally is formal.

CONCERNING THE WEATHER: Be sure to wear a suit and bring a top coat. The ocean fogs are cold and damp. If the weather measures up to the famous sunny California weather, summer clothes will be needed.

BOARD OF COUNSELLORS OF THE A.S.M.T.

District — *States*

NEW ENGLAND—Maine, New Hampshire, Vermont, Massachusetts, Rhode Island, Connecticut.
Counsellor—Miss Lily Ford, 9 Sixth St., Dover, New Hampshire.

NORTH ATLANTIC—New York, New Jersey.

MIDDLE ATLANTIC—Delaware, Maryland, Pennsylvania, Washington, D. C.
Counsellor—Miss Mollie Hill, 2325 37th St., N.W., Washington 7, D.C.

SOUTH EASTERN—Virginia, West Virginia, North Carolina, South Carolina.
Counsellor—Miss Ida Reilly, Roanoke Hospital Association, Roanoke 4, Virginia.

SOUTHERN—Georgia, Alabama, Kentucky, Tennessee.
Counsellor—Mrs. Mary L. Childress, 654 So. 34th St., Louisville, Ky.

GULF STATES—Florida, Mississippi, Louisiana.
Counsellor—Miss Lucille Godelfer, 2928 Bell St., New Orleans, La.

SOUTH CENTRAL—Arkansas, Oklahoma, Missouri, Kansas.
Counsellor—Miss Ann Snow, 216 E. D Ave., Park Hill, North Little Rock, Arkansas.

GREAT LAKES—Ohio, Michigan, Indiana.
Counsellor—Miss Louise Vance, 105 So. York St., Elmhurst, Ill.

MIDDLE WESTERN—Wyoming, Colorado, Nebraska, Montana.
Counsellor—Miss Bernice Elliott, 5107 Webster St., Omaha 3, Neb.

NORTH CENTRAL—Minnesota, Wisconsin, North Dakota, South Dakota, Iowa.
Counsellor—Miss Frieda Claussen, 469 Laurel Ave., St. Paul, Minn.

NORTH WESTERN—Washington, Oregon, Idaho.
Counsellor—Miss Clara Becton, 2851 N. Vancouver Ave., Portland, Oregon.

WESTERN—California, Nevada, Utah.

SOUTH WESTERN—Arizona, New Mexico, Texas.
Counsellor—Mr. Paul Lea, Lubbock Clinic and Hospital, Lubbock, Texas.

FOREIGN—Counsellor—Miss Mary Connor, Queen's Hospital, Honolulu, I. H.

*SAY IT IN FIGURES

Through the courtesy of the offices of the Executive Secretary of the ASMT, the Registry (ASCP), and from the March, '45 report of the AMA's Committee on Medical Education and Hospitals the following figures are available:

Summary of Membership by States†

	ASMT	MT (ASCP)	AMA
Arkansas	23	91	130
Alabama	18	190	226
Arizona	6	45	92
Calofirnia	97	618	1,242
Colorado	23	233	181
Connecticut	10	224	190
Delaware	9	22	30
District of Columbia	15	100	109
Florida	41	168	436
Georgia	9	168	356
Idaho	10	30	40
Illinois	112	554	823
Indiana	46	235	270
Iowa	24	99	153
Kansas	28	207	173
Kentucky	40	212	181
Louisiana	23	164	228
Maine	7	44	71
Maryland	20	149	316
Massachusetts	35	244	638
Michigan	41	447	419
Minnesota	92	350	216
Mississippi	10	59	193
Missouri	51	312	382
Montana	10	70	38
Nebraska	22	99	113

*Reprinted from *The Arrow*, publication of th :Arrowhead Society of Medical Technologists, Duluth, Minn.

†Working full time at medical technology.

Nevada	3	11	24
New Hampshire	17	51	53
New Jersey	33	231	399
New Mexico	9	34	89
New York	75	547	1,643
North Carolina	21	272	340
North Dakota	9	46	41
Ohio	51	429	558
Oklahoma	49	127	191
Oregon	17	134	115
Pennsylvania	109	682	806
Rhode Island	1	20	66
South Carolina	6	80	224
South Dakota	12	28	53
Tennessee	27	158	192
Texas	115	580	912
Utah	6	51	80
Vermont	2	17	24
Virginia	27	240	357
Washington	19	199	264
West Virginia	13	74	163
Wisconsin	56	300	238
Wyoming	3	15	23
Foreign	32	238	?

The Registry has not included the newest registrants nor those withdrawn for various reasons, but it does include those delinquent in payment of dues, so that the Registry figures are approximately correct. The discrepancy between the numbers registered and the numbers active in the field are reported by the AMA seems to lie in the fact that many former medical technologists continue to register even after married in order to be available in case of emergency. With the exception of a few cases it is to be noted that the sea coast states show a greater number of medical technologists actively engaged in medical technology than are registered.

To all medical technologists the figures are revealing and to the pioneering medical technologists they are convincing that more HARD WORK is indicated.

STATE AND LOCAL SOCIETIES

TRI-STATE HOSPITAL ASSEMBLY

May 1, 2, 3, 1946—Illinois Society of Clinical Laboratory Technicians, together with Chicago Society of Medical Technologists and Wisconsin Society of Medical Technologists.

May 1, Palmer House, Dining Room 14, 3:45 to 5:45. Presiding, Helen Sunderlin. Role of the Medical Technologist in the Blood Bank. I. Davidsohn, M.D., Mt. Sinai Hospital, Chicago, Purpura. Steven C. Schwartz, M.D., Hematologist, Hektoen Institute. Streptomycin: A Survey of Its Origin, Nature and Properties. Louis M. Pruess, Ph.D., Research Department, Lederle Laboratories, Inc. Laboratory Diagnosis of Virus Diseases. H. J. Shaughnessy, Ph.D., Chief, Division of Laboratories, Illinois Department of Public Health.

May 2, Dining Room 17, 1:30 to 3:30. Presiding, Cecelia Kortuem. Incidence and Possible Pathogenicity of Endamoeba Fragilis. Mrs. Elda Knoll and Dr. Katherine Howell, Michael Reese Hospital. Laboratory Diagnosis of Salmonella Infections in the Small Laboratory. Oscar Felsenfeld, M.D., Mt. Sinai Research Foundation, Chicago. Clinical Laboratory Methods in Connection with the use of Anti-Biotics. Walter Priest, M.D., Associate in Medicine, Northwestern University Medical School. Liver Function Tests. Wm. S. Hoffman, M.D., Hektoen Institute.

May 3, Dining Room 17, 1:30 to 3:30. Presiding, Ethyl Trenary. The Anemias. Harold E. Marsh, M.D., Internist, Hackson Clinic, Madison, Wis. Distribution of the Rh Factor in Patients with Malignancies. Joseph F. Kuzma, M.D., Director of Laboratories, Milwaukee Hospital, Milwaukee. Post-War Medical Technology. Board of Registry of Medical Technologists of The American Society of Clinical Pathologists.

ARKANSAS SOCIETY OF MEDICAL TECHNOLOGISTS

Affiliated with A.A.S.M.T.

Meetings: Annually. Time: September

OFFICERS 1946

Miss Irrovia Corry, M.T. (ASCP) ASMT.....President
St. Vincent's Infirmary, Little Rock, Arkansas

Mrs. Elizabeth Burt, M.T. (ASCP).....Vice-President
Johnson and Ketz Clinic, Batesville, Arkansas

Miss Nicole Baird, M.T. (ASCP).....Secretary-Treasurer
814 E. 10th St., Little Rock, Arkansas

TEXAS

HARRIS COUNTY SOCIETY OF MEDICAL TECHNOLOGISTS

Not Affiliated with

AMERICAN SOCIETY OF MEDICAL TECHNOLOGISTS

Regular Meetings: Monthly

OFFICERS

Mary A. Nafai, M.T. (ASCP).....President

Aline Fischer, M.T. (ASCP).....Vice-President

Doris Wallace, M.T. (ASCP).....Secretary-Treasurer

Vondell Stewart, M.T. (ASCP).....Program Chairman

Meetings of the Harris County Society of Medical Technologists are held on the third Tuesday of each month. Their meetings for the past year have included a lecture by Dr. S. Knittel on "Anemias" and Dr. Louis Cope's talk on "Bone Marrow Studies" as well as their business meetings and Executive Staff meetings.

The guest speaker for February will be Dr. J. H. Gast, and the subject for the April meeting is "Laboratory Procedures for CO_2 Combining Power of Blood Plasma" by Dr. Eby Nell McElrath.

MADISON SOCIETY OF MEDICAL TECHNOLOGISTS

Affiliated with

WISCONSIN ASSOCIATION OF MEDICAL TECHNOLOGISTS

Regular Meetings: Monthly

OFFICERS

Betty Johnson (ASCP) ..... President
Katherine Rupp (ASCP) ..... President-elect
Florence Griswold (ASCP) ..... Treasurer
Betty Joseph (ASCP) ..... Secretary
Wisconsin General Hospital, Madison, Wisconsin

A dinner is planned for May 8th at the Capitol Hotel, at which Dr. J. Pasternak, of St. Mary's Hospital, will be the speaker. The guests of this meeting will be the doctors who have spoken at the monthly meetings during the year.

WISCONSIN ASSOCIATION OF MEDICAL TECHNOLOGISTS

Affiliated with

AMERCAN SOCIETY OF MEDICAL TECHNOLOGISTS

Regular Meetings: Semi-annually

Ethel Trenary (ASCP) ..... President
Geralden Miller (ASCP) ..... President-elect
Sylvia V. Anderson (ASCP) ..... Secretary
8020 Harwood Avenue, Milwaukee
Esther Lemont (ASCP) ..... Treasurer

The next event of this society will be the spring convention which will be held in joint session with the Tri-State Hospital Assembly, at the Palmer House in Chicago, Illinois, on the 3rd of May. Two hours of special interest for Medical Technologists is scheduled for Wisconsin Day, a business meeting following, and a dinner concluding the session.

TEXAS

TARRANT COUNTY SOCIETY OF MEDICAL TECHNOLOGISTS

Not Affiliated with

AMERICAN SOCIETY OF MEDICAL TECHNOLOGISTS

Regular Meetings: Monthly

OFFICERS

Mrs. Ruby Lee Oxford, M. T.....President
Mrs. Edith Mazurek, M. T.....Vice-President
Miss Mary Jean Guthrie, M. T.....Secretary-Treasurer
Miss Dorothy Patras, M. T.....Corresponding Secretary

The Tarrant County Society of Medical Technologists hold their monthly meetings on the first Thursday of each month.

MICHIGAN

From now on the Ferguson-Droste-Ferguson Sanitarium of Grand Rapids, Michigan, will be known as the birthplace of the Michigan Society of Medical Technologists. For on April 13, 1946, the body of an Michigan organization started taking form under the able counseling of Miss Louise Vance, 105 South York Street, Elmhurst, Illinois (Great Lakes Counsellors Board of the A.S.M.T.)

The chairman of this initial meeting was Miss Gladys Jacobs, of Bay City. Other Michiganites who attended the meeting were:

Miss Edna Luneke, Grand Rapids, Michigan
Miss Helen Psik, Jackson, Michigan
Miss Harriett Sadowski, Detroit, Michigan
Miss Mary Catherine Wethington, Detroit, Michigan
Mrs. Mildred Snover Zuidema, Port Huron, Michigan

Bay City, Michigan, June 8th, 1946, is the date set for the organizational meeting. All registered medical technologists in Michigan are urged to attend this meeting. The tentative program is as follows:

Afternoon, to be devoted to a scientific program,

Followed by a business session at which time the tentative constitution which was drawn up on April 13th, 1946, will be presented for approval and officers will be elected to pilot the Michigan

Society of Medical Technologists to the place in the great organization Society of Medical Technologists to the place in the great origination of the American Society of Medical Technologists that most States in this our United States already are clamoring for.

The program will be climaxed by a banquet in the evening.

Congratulations, Michigan. Cooperation is the key to successful organization.

Submitted by

CECELIA M. KORTUEM, R.N., M.T. (ASCP)
Editorial Staff.

MINNESOTA

Minnesota is proud to announce that she has five medical technologists functioning in the presidential capacity in the state, each representing a district as follows:

District	No. I	Miss May Berntson, Bemidji
	No. II	Miss Elizabeth Hudberg, Duluth
	No. III	Miss June Tyson, Brainerd
	No. IV	Miss Dorothy Magaw, St. Paul
	No. V	Mrs. Vivian Wolffe, New Ulm

The Minnesota Hospital Association and its allied organizations convene in St. Paul, May 26, 1946. A panel discussion centering about the theme, "A Challenge of the Future," will highlight the general get-together feature of the 11 allied organizations.

Dr. Kano Ikeda addressed District No. 2 (Arrowhead Society of Medical Technologists) on Saturday, April 27, 1946, in Rockhurst Auditorium of the College of St. Scholastica, Duluth. His topic was, "The Future of Medical Technology." The society wishes publicly to thank Dr. Ikeda for his splendid talk and his genuine interest in medical technology.

WANTED: Technologist. Pediatricians office. Contact Conway Magee, M.D., Gordon Building, Lake Charles, La.

ARKANSAS

Society Name	Chartered	Meetings	Secretary's Address
Arkansas Society of M. T. Pres.—Irrovia Corry, M. T.	National	Annual	Miss Nicole Baird, M. T. 814 East 10th Street Little Rock, Arkansas
Little Rock Society of M. T. Pres.—Lila Church, M. T.	State	Quarterly	Mrs. Chas. Wilkens, M. T. Baptist State Hospital N. Little Rock, Ark.

CALIFORNIA

Santa Barbara Chapt. of M. T. Pres.—M. Royal, M. T. (ASCP)	None	Monthly	Mary C. Ryan, M. T. Santa Barbara Gen. Hospital Santa Barbara, California

COLORADO

Colorado Soc. of M. T. Pres.—Edna Mains, M. T.	National	Monthly	L. Ruth Robinson, M. T. 2389 Forest Street, Denver, Colo.

DISTRICT OF COLUMBIA

District of Columbia Society of M.T. Pres.—Zanerian E. Funk	National	Monthly	Evelyn F. Ballou, M.T. 4105 Third Street N.W. Washington, D. C.

GEORGIA

Savannah Society of M.T. Pres.—Sadie Cartwright	National	Monthly	Jurelle S. Hooper, M.T. 20 East 56th Street Savannah, Georgia

ILLINOIS

Illinois Society of Clinical Laboratory Technicians Pres.—Fannie Warnock	National	Semi-annual	Edna H. Murmann, M.T. 3924 N. Monticello Ave. Chicago, Illinois
Chicago Society of M.T. Pres.—Helen E. Sunderland	National	Monthly	Joyce James, M.T. 939 North LaSalle Street Chicago 10, Illinois

INDIANA

Indiana State Society of M.T. Pres.—Virginia Sue Alley	National	Annual	Hazel Childs, M.T. Indianapolis City Hospital Indianapolis, Indiana
Indianapolis Society of M. T. Pres.—Mrs. Earl Lynn	National	Monthly	Miss Helen Kottolowski 1230 Villa Ave. Indianapolis, Indiana

KENTUCKY

Kentucky Society of M.T.	National	Monthly	Dorothea Distler, M.T. 860 Eastern Parkway Louisville, Kentucky